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MEETING REPORT

International Meeting "Immunotherapy of Cancer: Challenges and Needs"

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Abstract The main aims of the international meeting "Immunotherapy of Cancer: Challenges and Needs" were to review the state of the art of cancer immunotherapy and to identify critical issues which deserve special attention for promoting progress of research in this field, with a particular focus on the perspectives of clinical research. Novel concepts and strategies for identifying, monitoring and predicting effective responses to cancer immunotherapy protocols were presented, focused on the use of adjuvants (CpG oligonucleotides) or cytokines (IFN-alpha) to enhance the efficacy of cancer vaccines. Moreover, the possible advantages of using different types of dendritic cells (for active immunization strategies) or T cells (for adoptive immunotherapy protocols) were debated. A *consensus* was achieved on the need for enhancing the efficacy of cancer vaccines or adoptive cell immunotherapy by combining these strategies with other anticancer treatments, including chemotherapy. Finally, initiatives for promoting clinical research by establishing a strategic cooperation in the field of cancer immunotherapy based on the active participation of all the relevant actors, including public institutions responsible of Public Health, National Cancer Institutes, industry, representatives of regulatory bodies, and patients' organizations were proposed.

Keywords Cancer · Vaccines · Immunotherapy

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Cancer vaccines: where are we now?

A general overview on the state of the art of anticancer vaccine trials was provided by Giorgio Parmiani (Milan), identifying critical reasons for the limited responses and making recommendations for future research. Overall, clinical responses range between 5 and 20%. Trials of vaccines containing dendritic cells (DC), showed a higher frequency of T lymphocyte responses than with peptide-based vaccines and adjuvants, but this was not consistently paralleled by an increase in the clinical response. Concerning the choice of tumor-associated antigens (TAAs), Parmiani critically reviewed the use of "self" TAAs, such as MelanA/MART-1 and CEA, illustrating how recognition of these antigens increases concomitantly with tumor burden. In contrast, the potential advantage of using unique TAAs was highlighted, based on the in vivo evidence of immune responses to this type of antigen in long-term surviving cancer patients. The identification of TAAs appropriate for preventing tumor immune escape was a recurring theme. The possibility of developing vaccines based on peptide epitopes derived from self-antigens and not presented by normal cells but only by processing-deficient cells (thereby acting as

immunogenic neoantigens targeted by CTL as demonstrated in mouse models [1]) was proposed by Rienk Offringa (Leiden). The potential of targeting oncoantigens (not easily down-modulated or negatively selected by immune attack) for prevention of tumor formation/progression was discussed by Federica Cavallo (Turin), who showed the effectiveness of vaccination strategies directed against HER-2 in mice transgenic for this oncogene. The novel cancer-testis antigen BORIS as a candidate for immunotherapy was proposed by Herbert C. Morse (Bethesda). BORIS is expressed very early in malignancy in a variety of cancers and induces the expression of many cancer-testis genes, including MAGE-A1 and NY-ESO-1. The antitumor efficacy of immunization with a DNA vaccine against BORIS together with CD80 was recently reported in a metastatic and poorly immunogenic murine mammary carcinoma model [2]. Thus, research on new TAAs remains an important area of investigation for the development of cancer vaccines.

The challenge of identifying, monitoring and predicting effective responses to cancer immunotherapy

The need for understanding the immunological basis of the failure or success of cancer immunotherapy as a critical step for the development of more effective cancer immunotherapy strategies is clear. Francesco Marincola (Bethesda) pointed out the importance of looking for the "immunological constant of rejection", i.e. the identification of common patterns underlying immune-mediated rejection at the tumor site. Serial gene expression analysis by cDNA microarrays of tumor biopsies led to the identification of events occurring during regression of basal cancer cell lesions treated with the TLR-7 agonist imiquimod (Marincola). Analysis of punch biopsies identified imiquimod-specific genes with effector functions (receptors and associated molecules as well as cytokines, cytokine receptors and lytic enzymes). Intriguingly, the same genes have been shown to be the most highly expressed during acute kidney rejection. Consistent with these observations is the association of organ-specific autoimmune-related adverse events to the clinical activity of a human anti-CTLA-4 antibody administered together with a vaccine, as illustrated by Jeffrey S. Weber (Los Angeles). Tumor cells can also suppress immune responses; for example, microvesicles released by human tumor cells or isolated from the plasma of melanoma patients may induce the generation of $CD14^+$HLA-DR-negative myeloid cells exerting TGF-β-mediated suppressive activity on lymphocyte proliferation and inducing down-modulation of cytotoxic molecules in activated $CD8^+$ T cells (Licia Rivoltini, Milan). These human suppressor cells may thus share some similarities with the mouse myeloid suppressor cells identified by Vincenzo Bronte (Padua) as a population of $CD11b^+$ inflammatory-type monocytes elicited by growing tumors and activated by IFN-γ released from T lymphocytes [3]. Reciprocally, the presence and role of a human counterpart of the mouse IKDC ($B220^+Ly6C^-NK1.1^+$ DC) identified by Laurence Zitvogel (Paris) as pivotal sensors and effectors of the innate anti-tumor immune response through their ability to secrete high levels of IFN-γ and mediate TRAIL-dependent cell lysis of tumor cells poorly recognized by NK cells [4] will be important to investigate.

A current major challenge is to identify correlates of protective vaccine-induced immune responses. Pierre Coulie (Brussels) pointed out that the frequencies of anti-melanoma $CD8^+$ T cells in the peripheral blood or in regressing melanoma lesions before and after anti-MAGE 3 vaccination suggest that it may be $CD8^+$ T cells specific for antigens other than those of the vaccine which mediate tumor regression. The anti-vaccine $CD8^+$ T cells that reach the tumor also undergo expansion and can then be detected in the blood. Thus, detection of anti-vaccine $CD8^+$ T cells in the blood may be the consequence of tumor destruction and not the pre-requisite for it. A novel ex vivo molecular-based approach at the single-cell level [5] has been developed to assess clonal composition of responding T cells and to track the dominant clonotypes over time during vaccination (Pedro Romero, Lausanne). Vaccine boosting of pre-existing dominant clonotypes, that were already present in melanoma patients (preceding vaccination), was documented in the differentiated effector-memory T cell compartment ex vivo. Overall, the data are consistent with a tumor-driven selection of the clonotypes followed by vaccine-driven boosting.

Strategies for enhancing the efficacy of cancer immunotherapy

Adjuvants

The identification of effective adjuvants may be critical for progress in vaccination against cancer. CpG plus IFA may be particularly potent (Romero), revealing induction of ex vivo-detectable Melan-A-specific $CD8^+$ T cells even in melanoma patients vaccinated with a peptide mixture containing the weak

native Melan-$A_{26–35}$ peptide. A recent clinical trial [6] aimed at evaluating the immune adjuvant effects of IFN-alpha administered with Melan-A/MART-1:26–35(27L) and gp100:209–217(210 M) peptides in melanoma patients showed enhancement of circulating $CD8^+$ T cells recognizing modified and native MART-1 and gp100 peptides and MART-1^+gp100^+ melanoma cells, along with an increased frequency of $CD45RA^+CCR7^-$ (terminally differentiated effectors) and $CD45RA^-CCR7^-$ (effector memory) cells (Maria Ferrantini, Rome). The IFN/vaccine treatment also resulted in a marked enhancement in the percentage of blood monocyte/DC precursors expressing high levels of costimulatory molecules. These data, along with a recently published study showing a direct correlation between enhanced survival in IFN-treated melanoma patients and development of autoimmunity [7], strongly support the concept that this cytokine can act as an effective cancer vaccine adjuvant. The use of different α-galactosylceramide (α-GalCer) analogues together with different TLR ligands can also represent a useful and potentially flexible method for inducing DC maturation through activation of iNKT cells (Vincenzo Cerundolo, Oxford). These results argue in favour of using compounds, which are weaker agonists than α-GalCer in clinical practice in order to minimize possible side effects caused by over-stimulation of iNKT cells.

Dendritic cells

In clinical trials involving about 100 melanoma patients vaccinated with tumor peptide-loaded monocyte-derived mature DC, both CTL and T-helper immune responses were clearly detected ex vivo in cohorts of patients vaccinated with multi-peptide DC vaccines with at least 10 million DC/injection/peptide class (Gerold Schuler, Erlangen). In these trials, a 31-month median overall survival was reached. However, in a randomized Phase III trial [8], no differences in the overall survival were observed in stage IV melanoma patients vaccinated with peptide-pulsed DC compared to the standard dacarbazine treatment, although a less advanced DC-based vaccine was used (i.e. fewer and less matured DC). Vaccination of metastatic melanoma patients with autologous DC-derived exosomes pulsed with different doses of MHC class I and II MAGE 3 peptides is effective in augmenting NK cells (Thomas Tursz, Villejuif). Exosomes from melanoma patients, but not their immature DC, contained functional NKG2D ligands and, after exosome treatment, restoration of NKG2D expression on circulating NK cells was observed, along with NK cell killing autologous tumor cells in vitro. The in vivo activation of the innate arm of immunity provides a novel indication for the potential application of DC-derived exosomes in cancer vaccination strategies. In melanoma patients immunized with mature DC pulsed with HLA class I and class II gp100/tyrosinase peptides, a correlation between the presence of tumor-specific T cells in DTH biopsies and progression-free survival or relapse-free survival was reported (Jolanda de Vries, Nijmegen). Thus, this type of immune monitoring should be considered in the design of vaccination studies in cancer patients.

Adoptive immunotherapy

The results of a phase I study of adoptive immunotherapy in metastatic breast cancer patients injected intravenously with bone marrow-derived memory T cells reactivated ex vivo by co-cultivation with antigen-pulsed DC was presented (Volker Schirrmacher, Heidelberg). Notably, this protocol resulted in the induction of immune responses in about 50% of the patients. The frequency of antigen-specific T cells detected in peripheral blood 7 days after transfer indicated that a massive expansion of the adoptively transferred cells occurred in vivo. A phase I study in melanoma patients receiving repeated i.v. infusions of in vitro expanded Melan-A-specific CTL also revealed an increase of circulating Melan-A-specific CTL from 0.01–0.07% up to 2% (Andreas Mackensen, Regensburg). An elevated frequency of Melan-A multimer+ T cells was demonstrated up to 14 days post transfer, suggesting long-term survival and/or proliferation of transferred CTL. ^{111}In-labeling of Melan-A-specific CTL demonstrated localization of transferred CTL to metastatic sites after injection. Overall, the results suggest that Melan-A specific CTL generated in vitro survive intact in vivo for several weeks and localize preferentially to tumor.

Combination therapies

Non-myeloablative chemotherapy can modulate the immune response and the antitumor activity of adoptively transferred immune lymphocytes (Paul F. Robbins, Bethesda). In melanoma patients treated with such chemotherapy and then given autologous tumor-reactive TILs, clinical responses were associated with the in vivo persistence of dominant TIL clonotypes. The in vivo therapeutic efficacy depended on proliferative potential and stage of differentiation of the transferred TIL [9]. A phase I/II study in prostate cancer patients randomized to receive either cyclophosphamide plus infusion of peripheral blood lymphocytes and vaccination

or vaccination only was presented (Bernard Fox, Portland). This treatment strategy, where patients are first rendered lymphopenic by treatment with chemotherapy and then reconstituted with autologous PBMCs before vaccine immunotherapy, aims at increasing the magnitude of the anti-tumor immune response obtained following vaccination and relies on the increased sensitivity to antigenic stimuli showed by lymphocytes placed under homeostasis-driven proliferation. A different strategy for counteracting negative regulatory mechanisms, by combining vaccination with anti-CTLA-4 treatment may also be effective (Jeffrey Weber, Los Angeles). Results of trials in melanoma patients receiving subcutaneous injections of MART-1/gp100/tyrosinase peptides in Montanide and intravenous injection of a humanised anti-CTLA-4 antibody indicated a dose-dependent induction of autoimmune-related adverse events, relapses in only 5 of 25 patients over a period of more than 12 months and high levels of immunity against MART-1 and gp100. Overall, these results indicate that blockade of CTLA-4 may disinhibit anti-tumor immunity which links autoimmunity and clinical response/benefit. Interesting correlations have been found between certain CTLA-4 single-nucleotide polymorphisms (SNPs) (JO30 and AG49) and autoimmunity, indicating that CTLA-4 SNPs may be useful for the prediction not only of autoimmunity but also clinical benefit in cancer immunotherapy. Weber proposed that new antibodies that alter immune regulatory pathways such as anti-PD-1, OX-40, and 41-BB should be rapidly moved into clinical trials. In addition, interesting strategies for enhancing cancer vaccine efficacy in mouse tumor models were provided by G. Ciliberto (use of TLR agonists, depletion of T regulatory cells) and by Mario P. Colombo (Milan), who showed the advantage of inactivating rather than depleting T regulatory cells by targeting selected molecules like GITR or OX40, in order to overcome the prompt recovery of T regulatory cells observed in tumor-bearing mice after mAb-mediated CD25 depletion. The results of a phase I/II trial in which ten stage II/IV melanoma patients with no evidence of disease were randomized to receive standard dacarbazine therapy alone or in combination with Montanide-emulsified Melan-A/MART-1:26–35(27L) and gp100:209–217(210 M) peptides (Enrico Proietti, Rome). Strong in vivo expansion of peptide-specific $CD8^+$ T cells was found in 4/5 patients treated with dacarbazine plus vaccine. These results suggest that the efficacy of cancer immunotherapy can be synergistically enhanced by combining cancer vaccines with conventional cancer treatment. Carl H. June (Philadelphia) illustrated how in the setting of lymphopenia, combined vaccine therapy and adoptive T cell transfer fosters the development of enhanced memory T cell responses. He reported the results of a phase I/II study in lymphopenic patients following high-dose chemotherapy and autologous hematopoietic stem cell transplantation. A single early post-transplant infusion of autologous T cells primed in vivo with a pneumococcal conjugate and costimulated ex vivo followed by post-transplant booster immunizations induced recovery from the severe immunodeficiency associated with high dose chemotherapy, and led to the induction of clinically relevant immunity.

Final remarks

New approaches are needed to face the global problem of cancer and cancer immunotherapy may represent an increasingly important strategy for cancer treatment, provided that some obstacles and barriers to progress in this field can be overcome (Peter Boyle, Lyon). The need for specific initiatives promoting and supporting clinical experimentation was an opinion commonly expressed by the participants. It was felt that it is timely and important to establish strategic cooperation among public health institutions, National Cancer Institutes, industry, representatives of regulatory bodies, and patients' organizations in this specific area of cancer research. This need is particularly felt by European researchers and clinicians, as the progress of European research in this field would greatly benefit from coordinated initiatives that promote clinical research, avoiding unnecessary duplication and fragmentation and favoring cooperation and harmonization of the regulatory framework for clinical studies (Alexander M.M. Eggermont, Filippo Belardelli, Stefano Vella, Michele Maio). These issues can be addressed in the framework of the EUROCAN + PLUS project, the "Feasibility Study for Coordination of National Cancer Research Activities" funded by the European Commission and coordinated by the IARC, Lyon. In order to overcome impediments posed by the current application to cancer vaccines of the conventional development paradigms developed for cytotoxic drugs, the definition of a new paradigm specifically for cancer vaccination is required. A *consensus* document addressing this issue has been elaborated by the Cancer Vaccine Clinical Trials Working Group [10] and will provide an important starting point for further discussion with regulatory authorities. A particular effort is required for initiatives promoting clinical research based on combination therapies, and this process has already been initiated in the US (Bernard

Fox, Portland). Addressing all these issues in a comprehensive manner will be instrumental to providing cancer immunotherapy the chance of fully demonstrating its potential of becoming an effective standard strategy for patient care.

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Appendix

The Meeting was held May 24–25, 2006, at the Istituto Superiore di Sanità (ISS), Rome, Italy. In addition to the organizers (E. Garaci, F. Belardelli, M. Ferrantini, F.M. Marincola, G. Parmiani, S. Vella), the speakers included Drs. J. Bartholeyns (IDM, Paris, France), P. Boyle (International Agency for Research on Cancer, Lyon, France), V. Bronte (Istituto Oncologico Veneto, Padua, Italy), R. Camerini (Sigma-Tau i.f.r. S.p.A., Rome, Italy), F. Cavallo (San Luigi Gonzaga Hospital, University of Turin, Italy), V. Cerundolo (Weatherall Institute of Molecular Medicine, Oxford, UK), G. Ciliberto (Istituto di Ricerche di Biologia Molecolare P. Angeletti, Pomezia, Rome, Italy), M.P. Colombo (Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy), P.G. Coulie (University of Louvain, Brussels, Belgium), I.J.M. de Vries (Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands), A.M.M. Eggermont (Erasmus University Medical Center, Rotterdam, The Netherlands), B. Fox (Earle A. Chiles Research Institute, Portland, OR, USA), C.H. June (University of Pennsylvania, Philadelphia, PA, USA), A. Mackensen (University of Regensburg, Germany), M. Maio (University Hospital of Siena, Italy), H.C. III Morse (National Institutes of Health, Bethesda, MD, USA), P. Nisticò (Regina Elena Cancer Institute, Rome, Italy), R. Offringa (University of Leiden, The Netherlands), E. Proietti (Istituto Superiore di Sanità, Rome, Italy), L. Rivoltini (Istituto Nazionale Tumori, Milan, Italy), P.F. Robbins (National Cancer Institute, Bethesda, MD, USA), P. Romero (Institute for Cancer Research, Lausanne, Switzerland), V. Schirrmacher (German Cancer Research Center, Heidelberg, Germany), G. Schuler (University Hospital, Erlangen, Germany), T. Tursz (Institut Gustave Roussy, Villejuif, France), J. Weber (University of Southern California, Los Angeles, CA, USA), L. Zitvogel (Institut Gustave Roussy, Villejuif, France).

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